Anticancer activity of some novel thieno [2, 3-d] pyrimidine derivatives.

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Abstract

As part of our search for searching for anticancer agents a novel series of thieno [2, 3-d] pyrimidine derivatives 9-14 were obtained via reaction of the strategic starting material ethyl 2-isothiocyanato-4, 5-dimethylthiophene-3-carboxylate 2 with sulfa-drugs namely, sulfanilamide, sulfathiazole, sulfadiazone, sulfamerazine, sulfadimethoxazine and sulfadoxine, in dimethylformamide containing triethylamine as catalyst. The structures of the newly synthesized compounds were established by microanalysis, IR, 1H-NMR, 13C-NMR and mass spectral data. All the newly synthesized compounds were evaluated for their in vitro anticancer activity against human breast cancer cell line (MCF7). Most of the screened compounds exhibited higher anti-breast cancer activity compared with Doxorubicin as a reference drug. Compounds 14, 13, 9 and 12 (IC50 values 22.12, 22.52, 27.83 and 29.22 µM) showed higher anti-breast cancer activity than the Doxorubicin as a reference drug with (IC50 value 30.40 µM). In addition, compounds 10 and 11 with (IC50 values 34.64, 37.78 µM) are nearly as active as Doxorubicin as positive control.

Keywords: Design, Synthesis, Thieno [2, 3-d] pyrimidines, Anti-breast cancer activity.

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Introduction

According to WHO population-based data, cancer is a leading cause of mortality worldwide accounting for almost 13% of all death [1]. Among all types of cancer, lung, breast, colorectal, stomach, and prostate cancer is the underlying cause for the majority of cancer death. Hitherto, chemotherapy remains one of the therapeutic strategies adopted worldwide for the management of cancer either alone or in conjunction with surgery and/or radiotherapy. Currently in clinical use anticancer agents suffer from a number of drawbacks correlated to drugs’ associated side effects and/or tumors’ multi-drug resistance [2,3]. Hence, it obviously is still of interest to search for new bioactive molecules having anticancer activity. Thiophenes and thienopyrimidines have been reported to possess interesting biological and pharmacological activities where several derivatives are used as antibacterial [1-3], anti-inflammatory [4], anticancer [5,6], and antiviral agents [7].

From the chemical and structural point of view, literature survey showed that sulfonamide [4-8], bearing molecules play an important role in the anticancer activity. In addition, various compounds with a heterocyclic backbone scaffold demonstrated promising anticancer activity. For example, a number of thienopyrimidine derivatives were claimed to possess interesting anticancer activities [9,10]. Sulfonamides anticancer activity has been in many instances attributed to inhibition of carbonic anhydrase enzymes [4-6]. Carbonic anhydrases (CA, EC 4.2.1.1) represent a family of Zn based metallo enzymes that catalyzes the interconversion between carbon dioxide and bicarbonate with generation of protons. The carbonic anhydrase isozyme IX (CA IX) is reported to be associated with tumorogenesis being highly over expressed in hypoxic tumors and restrictedly expressed in normal tissues [11-14]. CA IX inhibitors have been shown to display promising anticancer activity in addition to having fewer side effects compared to other anticancer drugs. Many research endeavors have reported sulfonamide bearing molecules as promising anticancer agents acting through inhibition of carbonic anhydrase IX [11-14]. Most cancer patients are subjected to chemotherapy for the treatment of advanced cancers. However, most metastatic solid tumors eventually remain incurable even by treatment with recent anticancer drugs. Also, Cancer is a disease of striking significance in the world today. Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutic is often limited mainly due to undesirable side effects and a
limited choice of available anticancer drugs [15-17]. This clearly underlines the urgent need for developing novel chemotherapeutic agents with more potent anticancer activities. Many anticancer agents which act as tyrosine kinase inhibitors comprised the pyrimidine nucleus as a core moiety. This could be exemplified by different quinazoline derivatives such as gefitinib (IressaTM) [18] and tandutinib (MLN518) (phase II clinical trials [19] (Figure 1). In continuation of our work [20-24], it seemed of interest to design and synthesize a novel series of thienopyrimidines bearing biologically active sulfonamide moieties, analogues to gefitinib (IressaTM) and tandutinib (MLN518) to evaluate their anti-breast cancer activity.

**Experimental**

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5 ml) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded on KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). 1H-NMR spectra (in DMSO-d$_6$) were recorded on Bruker AC-300 ultra-shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz, using TMS as internal standard. Electron impact Mass Spectra were recorded on a Shimadzu GC-Ms-Qp 5000 instruments (Shimadzu, Tokyo, Japan). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ± 0.4% of the theoretical values.

**Results**

**General Procedure for the synthesis of novel thienopyrimidine derivatives 9-14.**

A mixture of ethyl 2-isothiocyanato-4, 5-dimethylthiophene-3-carboxylate 2 (2.41 g, 0.01 mole), sulfa-drugs (0.012 mole) in dimethylformamide (20 ml) containing 3 drops of triethylamine was heated under reflux for 14 h. The reaction mixture was allowed to cool, filtered off the solid obtained and recrystallized from dioxane to give compounds 9-14, respectively.

**Synthesis of 4-(5, 6-dimethyl-4-oxo-2-thioxo-1, 2-di-, hydrothieno[2, 3-d]pyrimidine-3(4H)-yl)benzenesulfonamide(9).**

Yield, 86%; m.p. 279.0°C; IR (KBr, cm$^{-1}$): at 3437, 3425, 3263 (NH, NH$_2$), 3100 (CH arom.), 2978, 2947(CH aliph.), 1674(C=O), 1338, 1161(SO$_2$), 1234 (C=S). 1H-NMR (DMSO-d$_6$): 2.2, 2.3 [2s, 6H, 2CH$_3$], 7.3-8.2 [m, 6H, Ar-H + SO$_2$NH$_2$], 11.2 [s, 1H, NH, exchangeable with D$_2$O]. 13C-NMR (DMSO-d$_6$): 11.2, 12.4, 115.8, 120.4 (2), 128.8 (2), 130.7, 131.8, 133.9, 134.6, 148.2, 159.7, 180.1. MS m/z (%): 367 [M+] (20.18), 151 (100). Anal. Caled. for C$_{14}$H$_{13}$N$_3$O$_3$S$_3$: C, 45.76; H, 3.57; N, 11.44. Found: C, 45.48; H, 3.25; N, 11.12.

**Synthesis of 4-(5, 6-dimethyl-4-oxo-2-thioxo-1, 2-dihydrothieno[2, 3-d]pyrimidine-3(4H)-yl)-N-(thiazol-2-yl)benzenesulfonamide(10).**

Yield, 90%; m.p. 205.1°C; IR (KBr, cm$^{-1}$): 3383, 3375 (NH), 3078 (CH arom.), 2978, 2947(CH aliph.), 1654 (C=O), 1593(C=N), 1388, 1141(SO$_2$), 1238 (C=S). 1H-NMR (DMSO-d$_6$): δ: 2.2, 2.3 [2s, 6H, 2CH$_3$], 6.8-8.2 [m, 6H, Ar-H], 8.7 [s, 1H, SO$_2$NH, exchangeable with D$_2$O], 11.1 [s, 1H, NH, exchangeable with D$_2$O]. 13C-NMR...
Synthesis of 4-(5, 6-dimethyl-4-oxo-2-thioxo-1, 2-dihydrothieno [2, 3-d] pyrimidin-3(4H)-yl)-N-(pyrimidin-2-yl) benzenesulfonamide (11).

Yield, 84%; m.p. 195.3 [2, 6H, 2CH], 7.1-8.5 [m, 6H, Ar-H], 9.1 [s, 1H, SO2NH, exchangeable with D2O], 11.3 [s, 1H, NH, exchangeable with D2O]. 13C-NMR (DMSO-d6): δ: 10.7, 12.3, 123.4 (C=S). 1H NMR (DMSO-d6): 2.2, 2.3 [2s, 6H, 2CH], 7.1-8.5 [m, 6H, Ar-H], 9.1 [s, 1H, SO2NH, exchangeable with D2O], 11.3 [s, 1H, NH, exchangeable with D2O]. 13C-NMR (DMSO-d6): 10.7, 12.3, 23.5 (2), 117.7, 115.0, 120.6 (2), 128.7 (2), 129.4, 130.6, 132.8, 133.8, 150.0, 152.6, 157.7, 162.0, 182.6. MS m/z (%): 505 [M+H+] (7.56), 184 (100). Anal. Caled. for C25H23N3O3S: C, 47.84; H, 3.54; N, 13.49.

Synthesis of 4-(5, 6-dimethyl-4-oxo-2-thioxo-1, 2-dihydrothieno [2, 3-d] pyrimidin-3(4H)-yl)-N-(4, 6-dimethyl-pyrimidin-2-yl)benzenesulfonamide (12).

Yield, 83%; m.p. 209 [s, 1H, NH, exchangeable with D2O], 10.6 [s, 1H, NH, exchangeable with D2O]. 13C-NMR (DMSO-d6): δ: 11.2, 12.4, 55.2, 55.6, 81.7, 115.6, 120.2 (2), 127.0 (2), 129.6, 130.8, 132.9, 133.4, 150.1, 155.5, 158.7, 166.2, 169.8, 181.6. MS m/z (%): 505 [M+H+] (12.18), 153 (100). Anal. Caled. for C20H17N3O3S: C, 47.51; H, 3.79; N, 13.85. Found: C, 47.84; H, 3.54; N, 13.51.

(Scheme 1)

In vitro Anticancer Activity

The cytotoxic activity was measured in vitro for the newly synthesized compounds using the Sulforhodamine-B stain (SRB) assay using the Skehan et al. [25]. The in vitro anticancer screening was done at the Pharmacology Unit, the National Cancer Institute, Cairo University. Cells were plated in 96-multiwell microtiter plate (104-cells/well) for 24h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate concentration. Different concentrations of the compound under test (10, 25, 50 and 100 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (W/V) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an enzyme-linked immunosorbent assay ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line under the specified time [25]. The molar concentration required for 50% inhibition of cell viability (IC50) was calculated and the results are given in (Table 1). The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability.

In vitro Anti-Breast Cancer Activity

The newly synthesized compounds were evaluated for their in vitro anticancer activity against human breast cancer cell line, MCF7. Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the in vitro cytotoxic activity of the newly synthesized compounds. Most of the tested...
The compounds were designed in the aim of exploring their anti-breast cancer activity. The sequence of reaction followed in the synthesis of the target compounds is illustrated in (Scheme 1). As a part of a program aimed at the synthesis of novel thieno [2, 3-d]pyrimidine derivatives having the biologically active sulfonamide moieties 9-14, namely sulfanilamide 9, sulfa-thiazole10, sulfa-diazine11, sulfa-merazine12, sulfa-dimethoxazine13 and sulfa-doxine14, which could be useful for biological screening, we have investigated the possible utility of 2-isothiocyanatothiophene 2 [26] to react with sulfa-drugs in dimethylformamide in presence of trimethylamine as catalyst to give novel thienopyrimidine derivatives 9-14 in high yield (Scheme 1). Thus, treatment of 2 with sulfa-drugs in refluxing dimethylformamide in presence 

Table 1: In-vitro anticancer screening of the newly synthesized compounds against human breast cancer cell line (MCF-7)

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Compound concentration (µM)</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 (µM)</td>
<td>25 (µM)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.7211 ± 0.06</td>
<td>0.5463 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>0.8425 ± 0.07</td>
<td>0.5324 ± 0.06</td>
</tr>
<tr>
<td>11</td>
<td>0.9294 ± 0.09</td>
<td>0.6825 ± 0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.9128 ± 0.08</td>
<td>0.6316 ± 0.09</td>
</tr>
<tr>
<td>13</td>
<td>0.8334 ± 0.08</td>
<td>0.6153 ± 0.05</td>
</tr>
<tr>
<td>14</td>
<td>0.7656 ± 0.06</td>
<td>0.4462 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0.7474 ± 0.05</td>
<td>0.4813 ± 0.07</td>
</tr>
</tbody>
</table>

Discussion

The compounds were designed in the aim of exploring their anti-breast cancer activity. The sequence of reaction followed in the synthesis of the target compounds is illustrated in (Scheme 1). As a part of a program aimed at the synthesis of novel thieno [2, 3-d]pyrimidine derivatives having the biologically active sulfonamide moieties 9-14, namely sulfanilamide 9, sulfa-thiazole10, sulfa-diazine11, sulfa-merazine12, sulfa-dimethoxazine13 and sulfa-doxine14, which could be useful for biological screening, we have investigated the possible utility of 2-isothiocyanatothiophene 2 [26] to react with sulfa-drugs in dimethylformamide in the presence of trimethylamine as catalyst to give novel thienopyrimidine derivatives 9-14 in high yield (Scheme 1). Thus, treatment of 2 with sulfa-drugs in refluxing dimethylformamide in presence
of triethylamine as catalyst furnished the corresponding sulfonamide derivatives 9-14, through the formation of intermediates 3-8. The structures of the later products were assigned on the basis of their analytical and spectral data. The IR spectra of the reaction products showed in each case three absorption bands corresponding to NH functions in the region 3432-3110 cm\(^{-1}\), in addition to a carbynol absorption band in the region 1703-1654 cm\(^{-1}\)

| 3437 | 3425 | 3263 cm\(^{-1}\) absorption bands assigned to C=S function in the region 1244-1230 cm\(^{-1}\), absorption bands due to SO\(_2\) functions in the region 1388-1141 cm\(^{-1}\). IR spectrum of compound 9 revealed the absence of N=C=S group and presence of characteristic bands at 3425, 3124 cm\(^{-1}\), absorption bands assigned to C=S function in the region 1244-1230 cm\(^{-1}\), absorption bands due to SO\(_2\) functions in the region 1388-1141 cm\(^{-1}\). IR spectrum of compound 9 showed the absence of N=C=S group and presence of characteristic bands at 3425, 3124 cm\(^{-1}\). Compound 10 exhibited singlet at 180.1 ppm attributed to C=S group. Compound 10 was established on the basis of elemental analysis and spectral data. IR spectrum of compound 10 showed the absence of N=C=S group and presence of characteristic bands at 3432, 3124 cm\(^{-1}\) (NH), 3078 cm\(^{-1}\) (CH aliph.), 1654 cm\(^{-1}\) (C=O), 1593 cm\(^{-1}\) (C=N), 1342, 1161 cm\(^{-1}\) (SO\(_2\)), 1234 cm\(^{-1}\) (C=S). IR spectrum of compound 10 in (DMSO-d\(_6\)) revealed signals at 8.7, 11.1 ppm due to SO\(_2\)NH and NH groups. IR spectrum of compound 10 showed singlet at 178.4 ppm for C=S group. IR spectrum of compound 11 was proved on the basis of elemental analysis and spectral data. IR spectrum of compound 11 showed the absence of N=C=S group and presence of characteristic bands at 3432, 3124 cm\(^{-1}\) (NH), 3109 cm\(^{-1}\) (CH aliph.), 1685 cm\(^{-1}\) (C=O), 1577 cm\(^{-1}\) (C=N), 1342, 1161 cm\(^{-1}\) (SO\(_2\)), 1234 cm\(^{-1}\) (C=S). IR spectrum of compound 11 in (DMSO-d\(_6\)) revealed signals at 9.1, 11.3 ppm due to SO\(_2\)NH and NH groups. IR spectrum of compound 11 exhibited singlet at 182.0 ppm attributed to C=S group. Compound 11 was proved on the basis of elemental analysis and spectral data. IR spectrum of compound 11 showed the absence of N=C=S group and presence of characteristic bands at 3432, 3124 cm\(^{-1}\) (NH), 3109 cm\(^{-1}\) (CH aliph.), 2974, 2947 cm\(^{-1}\) (CH aliph.), 1685 cm\(^{-1}\) (C=O), 1597 cm\(^{-1}\) (C=N), 1381, 1161 cm\(^{-1}\) (SO\(_2\)), 1230 cm\(^{-1}\) (C=S). IR spectrum of compound 11 in (DMSO-d\(_6\)) showed signals at 2.4 ppm attributed to CH\(_2\) group for pyrimidine ring. IR spectrum of compound 11 revealed singlet at 181.8 ppm according to C=S group. Compound 12 was elucidated on the basis of elemental analysis and spectral data. IR spectrum of compound 12 revealed the absence of N=C=S group and presence of characteristic bands at 3340, 3186 cm\(^{-1}\) (NH), 3055 cm\(^{-1}\) (CH aliph.), 2941, 2836 cm\(^{-1}\) (CH aliph.), 1703 cm\(^{-1}\) (C=O), 1618 cm\(^{-1}\) (C=N), 1382, 1153 cm\(^{-1}\) (SO\(_2\)), 1244 cm\(^{-1}\) (C=S). IR spectrum of compound 12 in (DMSO-d\(_6\)) showed singlet at 3.8 ppm assigned to 2OCH\(_3\) groups. IR spectrum of compound 12 revealed singlet at 55.2, 55.6 ppm attributed to 2OCH\(_3\) groups. Compound 14 was elucidated on the basis of elemental analysis and spectral data.

**Conclusion**

The objective of the present study was to synthesize and investigate the anti-breast cancer activity of some novel thieno [2,3-d] pyrimidine derivatives carrying the biologically active benzenesulfonamide moieties at 3-position and thione moiety at 2-position. Compounds 14 bearing sulfa-doxine at 3-position, thione moiety at 2-position, 13 having sulfa-dimethoxazine, 9 carrying the corresponding sulfanilamide and 12 incorporating sulfamerazine with IC\(_{50}\) values (22.12, 22.52, 27.83, 29.22 µM) were found the most active compounds compared with Doxorubicin as reference drug, while compounds 10 and 11 with IC\(_{50}\) values (34.64, 37.78 µM) are nearly as active as Doxorubicin as positive control.

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**References**

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